REMARKS

Claims 1-9, 12, 14-27, 30-44, 47-57, 59-61, 64-74, 76-78, 81-90 and 93-96 were pending in this application prior to this response. By the present communication, no new claims have been added, claims 19-27, 30, 32-44, 47-57, 59-61, 64-74, 76-78, 81-86, 88, 89 and 93 have been cancelled without prejudice, and claims 1, 6-9, 12, 14, 31, 87, 90, 94, and 95 have been amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the Specification and original claims. Accordingly, claims 1-9, 12, 14-18, 31, 87, 90, 94-96 and 103 are currently pending in this application.

Objections to the claims

The Office Action indicates that claims 14-17, 32-35, 49-52, 66-69, 83-86, and 95-98 appear to encompass non-elected subject matter that allegedly involves "gene therapy." By the present amendment, these claims have either been cancelled without prejudice in response to the restriction requirement in this application or amended to delete reference to a "gene" so that claims to the subject matter of Group II can be addressed in a divisional application.

The Office Action also indicates that claims 9, 27, 41, 44, 61, 78 and 90 are objected to for including acronyms and claim 19 is objected to for missing a period. By the present communication, claims 27, 41, 44, 61 and 78 and 90 have been cancelled without prejudice and claims 9 and 90 have been amended to add the full name of the angiogenic cytokines referred to therein. Claim 19 has been amended to add the missing period.

In addition, the Examiner asserts that dependent claims referring to peripheral injection of autologous bone marrow aspirate (ABM) are broader than the parent claim, which is allegedly drawn to "trans-epicardial or endocardial injection" (Office Action, page 3). However, the Examiner may note that the parent claim actually recites "administration" of ABM to the heart or limb. Applicants respectfully submit that "administration" to a subject's the heart or limb can be accomplished endocardially (i.e., via a catheter) by peripheral injection

(e.g., into muscle tissue). Thus, the dependent claims objected to actually further limit the parent claim and are not broader than the parent claim.

However, to advance prosecution and limit the issues, Applicants have cancelled claims that refer to peripheral injection, thus rendering the objection to the claims moot on this point.

In view of the amendments, Applicants submit that the objections to the claims have been overcome and reconsideration and withdrawal of the objections are respectfully requested.

The Rejection under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully traverse the rejection of claims 6, 14-17, 24, 31, 39, 41, 48, 82 and 94 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. With regard to claim 6 and 24, the Examiner alleges that the term "limb" lacks antecedent basis. Claim 24 ha been cancelled, thus rendering the rejection moot as to claim 24. With regard to claim 6, Applicants submit that the phrase "heart or limb tissue" in claim 1 provides antecedent basis for the phrase "the limb tissue" in claim 6.

With regard to claims 14-17, the Examiner alleges that, as presently written, two separate selections appear to be required. To overcome the rejection and clarify the invention, claim 14 has been amended, as suggested by the Examiner, to replace the second use of the term "selected to" by the phrase "to promote endothelial cell proliferation, migration, or blood vessel formation," thus overcoming the grounds for the rejection.

With regard to claim 31, the Examiner asserts that base claim 19 lacks "antecedent basis" for the limitation "growing in culture." To clarify claim 31, Applicants have replaced the phrase "growing in culture" with the recitation: "further comprising culturing the autologous bone marrow aspirate prior to (b) to form conditioned medium containing bone marrow cells and injecting the conditioned medium into ischemic heart tissue." In view of the amendment, Applicants submit that any issue regarding lack of antecedent basis is overcome.

Similarly, with regard to claims 48, 82 and 94, the Examiner asserts that it is unclear whether the "conditioned medium" is the medium in which ABM cells are grown since the application does not define the term "derived from". Claims 48 and 82 have been cancelled without prejudice, rendering the rejection moot as to those claims, and claim 94 has been

amended to recite "conditioned medium in which autologous bone marrow cells have been grown", thus clarifying the origin and contents of the conditioned medium that forms an ingredient of the invention composition of claim 94. In addition, the phrase "is injected into ischemic heart muscle" has been deleted as being a method of use limitation that is inapposite for a composition claim.

In view of the amendments, Applicants submit that the pending claims meet all requirements under 35 U.S.C. § 112, second paragraph.

The Rejection Under 35 U.S.C. § 112, First Paragraph

Applicants traverse the rejection of claims 1-9, 12, 14-27, 30-44, 47-57, 59-61, 64-74, 76-78, 81-90 and 93-96 under 35 U.S.C. § 112, first paragraph as allegedly lacking enablement.

With regard to the Examiner's assertion that the application fails to provide enablement for stimulation of bone marrow aspirate using all kinds of stimulation, Applicants submit that the Specification teaches that "the bone marrow (BM) is a natural source of a broad spectrum of cytokines and cells that are involved in the control of angiogenic processes" (Specification, page 3, line 20; page 7, line 8). Applicants have used the terms "*'mixed' angiogenic cytokines*" (page 7, line 8) or "combined" agents (page 4, lines 10-11) to describe "a mixture of potent interactive growth factors that produce therapeutic angiogenesis and/or myogenesis" (page 7, lines 8-9). Thus, the idea that the application as filed describes stimulation of ABM only by MCP-1 is based on a lack of understanding of the invention.

Applicants have discovered that bone marrow can be stimulated ex vivo to produce this mixture of angiogenic cytokines by exposure to hypoxia or a form of energy or by ex vivo exposure to at least one of the disclosed angiogenic cytokines. Accordingly, Applicants teach that bone marrow aspirate can be stimulated to produce at least one of the endogenous mixture of angiogenic cytokines by culturing for a period of time sufficient to allow endogenous expression into the culture medium of one or more angiogenic cytokines. Alternatively, one or more angiogenic cytokines can be added directly *as proteins* to bone marrow aspirate to stimulate endogenous production of the mixture of angiogenic cytokines. Alternatively still, cells in bone marrow can be transfected with transgenes that express one or more of the identified angiogenic

cytokines and then cultured for a time sufficient to allow expression into the culture medium of transgene products, which will also stimulate production of endogenous angiogenic cytokines by bone marrow cells (See Specification, page 5, lines 5-14). Applicants have since discovered that paracrine signaling between vessel cells and cytokines is the mechanism involved.

Thus, Applicants disclose that ABM can be stimulated by a number of different ex vivo pathways; whereas previously it had been thought that bone marrow cells required stimulation in vivo (e.g., by injection into the donor of an angiogenic cytokine) prior to their collection. Accordingly, Applicants submit that limitation of ex vivo stimulation by exposure to a single cytokine would unjustifiably deprive Applicants of their right to a generic claim for ex vivo stimulation of ABM prior to injection of the ABM and for injection of cultured ABM and bone marrow-derived "products" (i.e., cultured and/or stimulated bone marrow cells and the cytokines expressed into the conditioned medium) for the purpose of treating heart or limb tissue in need of collateral blood vessel formation.

With regard to the Examiner's assertion that the claims lack enablement because injection of autologous bone marrow cells or stimulated autologous bone marrow cells would cause an immune response in the patient, Applicants submit that those of skill in the art would understand that the use of *autologous* bone marrow or cultured of *autologous* bone marrow products prevents immune response upon reinjection of the ABM or ABM culture medium because the patient's own cells produce the cytokines. Moreover, if the cells are stimulated by an angiogenic cytokine other than one produced in culture by the ABM itself, it is well known in the art that cytokines have a limited half-life and would be unlikely to persist in culture on the one hand, and, even if they did, do not raise the same risk of immune rejection as do large proteinaceous molecules. Moreover, in the case of myocardial infarction or ischemia, immune response to a beneficial cytokine is a calculated risk that is outweighed by the benefits. This type of calculated risk is now routinely undertaken in the treatment of life-threatening situations, such as certain cancers, even when that cancer has not reached an end stage.

Applicants further disagree with the Examiner's assertion that if the cell lineage is predetermined then treating a mixed population of ABMs with a stimulating factor may not achieve the desired outcome. Applicants have discovered that, in one embodiment, reinjection of

the stimulated bone marrow, including any products produced by the bone marrow after stimulation, provides an environment necessary for angiogenesis, such as development of collateral blood supply to ischemic tissue. Moreover, in another embodiment of the invention, Applicants have discovered that that administration of bone marrow cells after culture, in which case there is no longer a large mixture of cells, but only a few types (or perhaps even only one type), is also effective. Whether the ABMs contain committed stem cells or more primitive stem cells that have transdifferentiation ability is an interesting question, but is irrelevant to the claims since the Applicants do not seek to claim the mechanism, only the result. Applicants have discovered that the process involved in the invention method steps leads to successful transplant *and* subsequent growth of collaterals, irrespective of the state of differentiation of certain of the cells when the bone marrow is collected.

Applicants also wish to emphasize that this invention does not pertain to myogenesis *per se* in which it is hoped that injected cells will assume the phenotype of the tissue into they are injected, such as cardiac myocytes if injected into the myocardium. Rather the invention methods are based on the observation that bone marrow cells secrete numerous cytokines—an effect that can be augmented when the cells are exposed, in vitro, to hypoxia or an angiogenesis enhancing cytokine. When injected into ischemic tissue, these secreted cytokines then stimulate the growth and remodeling of blood vessels that are in the treated tissue, but are too small to result in substantial flow. In other words, this invention does not rely on transdifferentiation of the injected bone marrow cells into cells of the tissue into which they are injected, but relies instead upon the bone marrow cells surviving long enough (i.e., transient survival) to secrete cytokines that stimulate the formation of new blood vessels or result in the expansion of existing, but very small, blood vessels.

Since the filing of this application, Applicants have further tested the invention methods and compositions in the laboratory studies to prove its validity. Attached are copies of three publications by the inventors, and a published editorial that reviews and validates the above statements, one manuscript, one article that is in review at *Circulation*, and one additional manuscript that pertains to the gene therapy aspects of the invention, claims to which methods will be addressed in divisional and/or Continuation in Part applications now pending, which

applications describe the HIF-1 gene therapy studies. Taken together these documents corroborate the teachings of the Specification, and further illustrate the validity of the claimed methods and compositions.

Thus, Applicants disagree with the Examiner's assertion that the disclosure is enabling for direct administration to the heart, but is not enabling for administration elsewhere. Direct administration to the heart can involve intramuscular injection into heart tissue. Applicants teach that the invention methods for enhancing collateral blood vessel formation are equally effective when a composition comprising an effective amount of autologous bone marrow and/or bone marrow "products" (i.e., angiogenic cytokines or cells derived from culture of bone marrow such as the marrow-derived stromal cells discussed above) is administered to heart or limb tissue, for enhancing development of collateral flow in a patient in need thereof. Applicants submit that the Examiner has failed to present a rational basis for the conclusion underlying the rejection that blood vessels in heart tissue (i.e., myocardium) would be capable of developing into collateral blood vessels when treated using the invention methods and compositions, but blood vessels, such as immature blood vessels in peripheral limb tissue would not. Since the filing of the present application, studies have been published showing the restoration of blood flow in a chronic limb ischemia model upon administration of autologous bone marrow stromal cells (See Al-Khaldi A, et al., *Ann Thorac Surg.* 2003. 75:204-209, a copy of which is attached). In addition, the Examiner's particular attention is directed to the post-filing article by co-applicants S. Fuchs and S.E. Epstein describing studies in which cultured marrow-derived stromal cells with conditioned medium *or conditioned medium alone*, when obtained by culturing marrow-derived stromal cells under either normoxic or hypoxic conditions, enhanced collateral flow recovery and remodeling when injected into *murine hind limb ischemic tissue* (T. Kinnaird et al. 2004. *Circulation Research* March 19, 2004:678-685, a copy of which is attached). Applicants have elsewhere demonstrated that such stromal cells are obtained by culturing bone marrow, autologous or non-autologous (T.D. Kinnaird et al. "Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms." *Circulation*, in press)). ELISA analysis of the conditioned medium so

produced was shown to comprise a complex variety of endogenously expressed cytokines, with the marrow-derived stromal cells acting as tiny cytokine factories (See table , page 680).

This murine ischemic hind limb model study also demonstrated that bone marrow derived cells need not be incorporated into vessel structures to accomplish the therapeutic result. In fact, the conditioned medium alone was sufficient to promote development of collateral vessels and reverse the effect of limb damage by a mechanism described as paracrine signaling (Col 1, page 684). Accordingly, Applicants disagree with the Examiner's assertions that the applicant lacks enablement for the full scope of the present claims.

In view of the above, Applicants further disagree with the Examiner's assertion that the application is enabling only for stimulation of ABM with the cytokine MCP-1. However, claim 9 has been amended to place dependency upon claim 1, and now recites that the "composition further comprises VEGF and MCP-1. In the examples Applicants illustrate that culturing bone marrow results in secretion of enhanced levels of both VEGF and MCP-1, and these were the only two angiogenic cytokines that were measured by ELISA at the time of the filing (Specification, page 11, lines 12-22, See also Examples 1 and 2 and Fig. 1). As discussed above, Applicants' post-filing studies corroborate the teachings in the specification that a mixture of cytokines are secreted by bone marrow in vitro as well as in vivo. Thus Applicants submit that, at the least, enablement has been provided in this application sufficient for those of skill in the art to make and use the invention methods and compositions that incorporate the particular angiogenesis-enhancing cytokines VEGF and MCP-1, as recited in amended claim 9.

The Examiner has also asserted the claims lack enablement due to failure to recite an exact amount of the bone marrow therapeutic liquid to be administered per amount of ischemic tissue (Office Action, page 9). However, the invention methods as recited by amended claim 1 have been limited to administration to heart tissue (e.g., myocardium) for which Applicants have provided guidance regarding particulars of administration protocols, as acknowledged by the Examiner.

In addition, Applicants submit that, as in all therapeutic treatments, overdosage or underdosage are matters determined during clinical trials and are left to the purview of the Federal Drug Administration. Thus, Applicants recite "an effective amount" as is the custom in all therapeutic treatment claims and it is left to the physician to determine the "effective amount" based on such factors as the number of sites into which the therapeutic is administered, how closely spaced are the sites, the extent of ischemic tissue, the size and health of the patient, and the like.

Based on the above, Applicants respectfully submit that those of skill in the art are provided sufficient guidelines and information to make and use the claimed invention without undue experimentation. Applicants, therefore, respectfully request reconsideration and withdrawal of the rejection of claims under 35 U.S.C. § 112, first paragraph.

Regarding the Information Disclosure Statement

Applicants note that an Information Disclosure Statement (IDS) was mailed October 2, 2002, in connection with the subject application. However, an initialed copy of the form PTO-1449, indicating that the references cited therein were considered by the Examiner, has not been received. Also, only one of two pages of the form PTO-1449 mailed on July 9, 2002 has been received. Additionally, Applicants bring to the Examiner's attention the IDS mailed on April 6, 2004. Applicants respectfully request that the Examiner return an initialed copy of the above-mentioned form PTO-1449's, with the next Communication in this case. The Examiner is invited to contact Applicants' undersigned representative if copies of the IDS's and cited references are not readily available.

In view of the above amendments and remarks, Applicants submit that all rejections and objections have been overcome. Accordingly, reconsideration and favorable action on all pending claims are respectfully requested. If the Examiner would like to discuss any of the

issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Attachments:

1. Al-Khaldi et al., "Therapeutic angiogenesis using autologous bone marrow stromal cells: improved blood flow in a chronic limb ischemia model", *Ann Thorac Surg.* 2003 Jan; 75(1):204-9
2. Fuchs et al., "Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study", *J Am Coll Cardiol.* 2003 May; 41(10):1721-4
3. Fuchs et al., "Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia", *J Am Coll Cardiol.* 2001 May; 37(6):1726-32
4. Heil et al., "A different outlook on the role of bone marrow stem cells in vascular growth: bone marrow delivers software not hardware", *Circ Res.* 2004 Mar; 94(5):573-4
5. Kinnaird et al., "Bone-Marrow Derived Cells for Enhancing Collateral Development: Mechanisms, Animal Data, and Initial Clinical Experiences" (2004) *Circulation Research* 94:678-685.
6. Kinnaird et al., "HIV/VP16 Gene Therapy Enhances the In-Vitro and In-Vivo Angionenic/Arteriogenic Effects of Marrow-Derived Stromal Cells" *Circulation*, (in press)
7. Kinnaird et al, "Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms", *Circ Res.* 2004 Mar 19;94(5):678-85